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Title: METHOD AND APPARATUS FOR PERFORMING BIOLOGICAL REACTIONS ON A SUBSTRATE SURFACE

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METHOD AND APPARATUS FOR PERFORMING BIOLOGICAL REACTIONS ON A SUBSTRATE SURFACE

RELATED APPLICATIONS

This applications is a continuation-in-part of U.S. Application No. 09/464,490, filed December 15, 1999.

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to an apparatus for performing biological reactions. Specifically, the invention relates to an apparatus for performing nucleic acid hybridization reactions on a substrate layer having a multiplicity of oligonucleotide binding sites disposed thereon.

2. Description of the Prior Art

Recent advances in molecular biology have provided the opportunity to identify pathogens, diagnose disease states, and perform forensic determinations using gene sequences specific for the desired purpose. This explosion of genetic information has created a need for high-capacity assays and equipment for performing molecular biological assays, particularly nucleic acid hybridization assays. Most urgently, there is a need to miniaturize, automate, standardize and simplify such assays. This need stems from the fact that while these hybridization assays were originally developed in research laboratories working with purified products and performed by highly skilled individuals, adapting these procedures to clinical uses, such as diagnostics, forensics and other applications, has produced the need for equipment and

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methods that allow less-skilled operators to effectively perform the assays under higher capacity, less stringent assay conditions.

Nucleic acid hybridization assays are advantageously performed using probe array technology, which utilizes binding of target single-stranded DNA onto immobilized DNA (usually, oligonucleotide) probes. The detection limit of a nucleic acid hybridization assay is determined by the sensitivity of the detection device, and also by the amount of target nucleic acid available to be bound to probes, typically oligonucleotide probes, during hybridization.

Nucleic acid hybridization chambers are known in the prior art.

U.S. Patent No. 5,100,755 to Smyczek et al. discloses a hybridization chamber.

U.S. Patent No. 5,545,531 to Rava et al. discloses a hybridization plate comprising a multiplicity of oligonucleotide arrays.

U.S. Patent No. 5,360,741 to Hunnell discloses a gas heated hybridization chamber.

U.S. Patent No. 5,922,591 to Anderson et al. discloses a miniaturized hybridization chamber for use with oligonucleotide arrays.

U.S. Patent No. 5,945,334 to Besemer discloses oligonucleotide array packaging.

As currently employed, oligonucleotide array technology does not provide maximum hybridization efficiency. Existing nucleic acid hybridization assay equipment includes numerous components, each of which is a source of inefficiency and inaccuracy.

Hybridization using oligonucleotide arrays must be performed in a volume in which a small amount of target DNA or other nucleic acid can be efficiently annealed to the immobilized probes. For diagnostic assays, target DNA molecules are often obtained in minute (< picomol) quantities. In practice, it can take several (tens of) hours for hybridization to be substantially complete at the low target nucleic acid levels available for biological samples.

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In addition, array hybridization is conventionally performed in a stationary hybridization chamber where active mixing is absent. Under these conditions, the probability that a particular target molecule will hybridize to a complementary oligonucleotide probe immobilized on a surface is determined by the concentration of the target, the diffusion rate of the target molecule and the statistics of interaction between the target and the complementary oligonucleotide. Consequently, a larger number of samples must be tested to obtain useful information, and this in turn leads to increased hybridization times and inefficiencies.

In addition, efficiency is increased when the amount of user manipulation is kept to a minimum. As currently performed, oligonucleotide array hybridization requires a great deal of operator attentiveness and manipulation, and the degree of skill required to perform the analysis is high. For example, hybridization is typically performed in an assay chamber, and then data collection and analysis are performed in a separate apparatus (such as a laser scanner or fluorescence microscope). This arrangement requires a substantial amount of handling by the user, and makes the assays both time-consuming and subject to user error.

It is also a limitation of current practice that array hybridizations are performed one array at a time, thereby forgoing the economies of parallel processing and data analysis.

Additional limitations, inefficiencies, and expenses arise from the structural characteristics of existing apparatus. Many existing apparatus are limited in the size of the substrate they can accommodate. Other apparatus are not disposable and therefore require extensive cleaning between runs in order to prevent sample contamination. Yet other apparatus are high mass and therefore not susceptible of the rapid heating and cooling necessary for efficient hybridization. Other apparatus require the use of expensive optics for analysis of the reaction products.

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There remains a need in this art for an easy-to-use apparatus for performing biological reactions, particularly nucleic acid hybridization, that comprises a small reaction volume, where the fluid components can be actively mixed, that can be performed in parallel and that minimizes user intervention. There also remains a need for such an apparatus that is easy to manufacture in various sizes, that is disposable to minimize sample contamination, that allows for the use of low cost optical analytical equipment, and that is low mass to allow for rapid heating and cooling of the sample fluid. There also remains a need for methods for using such apparatus to increase hybridization efficiency, particularly relating to biochip arrays as understood in the art. This need is particularly striking, in view of the tremendous interest in biochip technology, the investment and substantial financial rewards generated by research into biochip technology, and the variety of products generated by such research.

SUMMARY OF THE INVENTION

The invention provides an apparatus in which biological reactions such as nucleic acid hybridization can be performed. The apparatus of the invention is a hybridization chamber comprising a flexible layer attached to a biochip by an adhesive layer. The biochip comprises a substrate having a first surface and a second surface, wherein the first surface contains a multiplicity of biologically reactive sites disposed in an area bounded by the adhesive layer set down on the first substrate surface. In a preferred embodiment, the multiplicity of biologically reactive sites comprises an oligonucleotide array. The flexible layer most preferably is both deformable and translucent. The adhesive layer, the flexible layer and the substrate also define a volume that is filled with a water-soluble compound. The water-soluble compound is preferably

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a solid at certain temperatures, most preferably at or below room temperature, and a liquid at elevated temperatures, most preferably below about 100°C.

The apparatus of the invention has first and second ports extending through the flexible layer into the chamber, rather than first and second ports extending through the substrate. In this embodiment, the apparatus comprises a substrate having a first surface, a flexible layer affixed to the first surface by a first adhesive layer, wherein the adhesive layer bounds an area on the surface of the substrate, first and second ports extending through the flexible layer into the area bounded by the first adhesive layer, and a second adhesive layer surrounding the first and second ports, wherein the second adhesive layer creates a seal between a pipette and the flexible layer. In particular embodiments of the apparatus, the second adhesive layer comprises a circle of adhesive surrounding each port having an inner diameter equal to the outer diameter of the port and an outer diameter larger than the outer diameter of the port. In an alternate preferred embodiment, the second adhesive layer covers the entire flexible layer except for those portions of the flexible layer through which the ports extend. In this embodiment, the apparatus further comprises a label layer affixed to the second adhesive layer, wherein the label layer has one or more windows corresponding in shape and size to the areas on the substrate surface bounded by the first adhesive layer.

In certain embodiments of the invention, the substrate comprises a multiplicity of oligonucleotide arrays, which are contained in one or a plurality of areas bounded by the adhesive layer and covered by the flexible layer. In these embodiments, each area bounded by the adhesive layer also comprises a first port and can also comprise a second port. In a further preferred embodiment, the oligonucleotides are bound to an array of anchoring structures. In a

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preferred embodiment, the anchoring structures comprise gel pads. In a further preferred embodiment, the anchoring structures comprise polyacrylamide gel pads.

The hybridization chamber is optionally supplied with a heater, most preferably a resistive heater, in thermal contact with the flexible layer. The chamber is also optionally supplied with a roller, most preferably a patterned roller, positioned in contact with the flexible layer and movable longitudinally across the surface of the chamber for mixing hybridization fluid and wash solutions as required. The chamber is further optionally supplied with a scanner positioned in contact with the flexible layer and movable longitudinally across the surface of the chamber for detecting hybridization signals, most preferably produced by fluorescently-labeled target nucleic acid.

In a further preferred embodiment, the invention provides a handheld device comprising a lid, a base, a carriage embodying a roller, a scanner, a heating element and a thermocouple, wherein the hybridization chamber of the invention is positioned above and in removable contact with the carriage.

Specific preferred embodiments of the present invention will become evident from the following more detailed description of certain preferred embodiments and the claims.

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DESCRIPTION OF THE DRAWINGS

Presently preferred embodiments of the invention are described with reference to the following drawings.

FIGS. **1A-1D** are views of a preferred embodiment of the present invention illustrating the preparation of a chamber for reaction. FIG. **1A** is a cross-sectional view of the apparatus illustrating a hybridization chamber prefilled with a water-soluble compound in thermal contact with a heating element. FIG. **1B** is a cross sectional view of the apparatus illustrating the mixing of the water-soluble compound and the biological sample fluid. FIG. **1C** is a cross sectional view of the apparatus illustrating a chamber filled with the sample fluid/water-soluble compound mixture, wherein the first and second ports are covered with a seal. FIG. **1D** is a top plan view of the apparatus illustrating the pattern of adhesive defining the individual areas containing the arrays of oligonucleotide probes.

FIG. **2** is an exploded cross-sectional view of a chamber showing the array of gel pads of a preferred embodiment of the invention.

FIG. **3** is an exploded perspective view of the array of biomolecular probes showing the positioning of the gel pads on the substrate of a preferred embodiment of the invention.

FIG. **4** is an exploded cross-sectional view of a port illustrating the conical shape of the port of a preferred embodiment of the invention.

FIG. **5** is a perspective view of the label layer, the flexible layer and the adhesive layer of a preferred embodiment of the invention.

FIG. **6** is a cross-sectional view of a stack of chambers according to a preferred embodiment.

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FIGS. **7A-7E** are top views of the layers of an alternate preferred embodiment of the invention having inlet and outlet ports extending through the flexible layer. FIG. **7A** is a view of the first adhesive layer, FIG. **7B** is a view of the flexible layer, FIG. **7C** is a view of the second adhesive layer, FIG. **7D** is a view of the label layer, and FIG. **7E** is a view of the layers of **7A** to **7D** as assembled.

FIGS. **8A-8B** are detail views of the notches cut into the first adhesive layer and the label layer of a preferred embodiment of the invention having inlet and outlet ports extending through the flexible layer.

FIGS. **9A-9C** are cross-sectional views of a preferred embodiment of the present invention illustrating the process of analyzing the array after completion of the reaction. FIG. **9A** shows the apparatus upon completion of the reaction. FIG. **9B** illustrates removal of the sample fluid from the chamber such that the flexible layer contacts the array. FIG. **9C** illustrates use of a laser scanner to analyze the array.

FIGS. **10A-10C** illustrate a handheld embodiment of the present invention. FIG. **10A** is a side view of the hand held scanning system. FIG. **10B** is a perspective view of a preferred embodiment comprising a hand-held scanning device illustrating the contact of the flexible layer with the carriage. FIG. **10C** is a view of the handheld system illustrating the lid containing the display unit.

FIG. **11A-11E** are cross-sectional views of the direct contact fiber optic scanner as shown in FIG. **10**.

FIG **12A-12C** are alternate embodiments illustrating the apparatus coupled to a sample preparation chip. FIG. **12A** illustrates an embodiment wherein the sample preparation chip is removably positioned against the second surface of the substrate. FIG. **12B** illustrates an

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embodiment wherein the sample preparation chip is affixed to the second surface of the substrate. FIG. **12C** illustrates an embodiment wherein the sample preparation chip is incorporated into the substrate.

FIG. **13** illustrates the assembly and use of a preferred embodiment of the present invention.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention provides an apparatus for performing high-capacity biological reactions on a biochip comprising a substrate having an array of biological binding sites. The invention provides a hybridization chamber having one or more arrays, preferably comprising arrays consisting of gel pads and most preferably comprising arrays consisting of 3-dimensional polymeric gel pads, wherein nucleic acid hybridization is performed by reacting a biological sample containing a target molecule of interest with a complementary oligonucleotide probe immobilized on the biochip. In preferred embodiments, the array is an oligonucleotide array comprising at least about 400, more preferably at least about 1000 and most preferably at least about 10,000 oligonucleotide probes. In these embodiments, the apparatus is most preferably used for nucleic acid hybridization of target nucleic acids in a biological sample, wherein the biological samples include, but are not limited to, nucleic acids, including DNA, genomic DNA, cDNA, and RNA.

As used herein, the term "array" refers to an ordered spatial arrangement, particularly an arrangement of immobilized biomolecules or polymeric anchoring structures.

As used herein, the term "addressable array" refers to an array wherein the individual elements have precisely defined x and y coordinates, so that a given element can be pinpointed.

As used herein, the term "biomolecular probe" refers to a biomolecule used to detect a complementary biomolecule. Examples include antigens that detect antibodies, oligonucleotides that detect complimentary oligonucleotides, and ligands that detect receptors. Such probes are preferably immobilized on a substrate.

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As used herein, the term "oligonucleotide probe" refer to a nucleic acid sequence used to detect the presence of a complementary target sequence by hybridization with the target sequence.

As used herein, the term "biochip" refers to a multiplicity of biomolecular probes, preferably oligonucleotide probes, immobilized on a substrate. In preferred embodiments, the multiplicity of biomolecular probes is provided as an ordered array; however, random placement of the multiplicity of probes is an available alternative.

The invention is advantageously used for performing assays using biochips **18**. Biochips, as used in the art, encompass substrates containing arrays or microarrays, preferably ordered arrays and most preferably ordered, addressable arrays, of biological molecules that comprise one member of a biological binding pair. Typically, such arrays are oligonucleotide arrays comprising a nucleotide sequence that is complementary to at least one sequence expected to be present in a biological sample. Alternatively, peptides or other small molecules can be arrayed in such biochips for performing immunological analysis (wherein the arrayed molecules are antigens) or assaying biological receptors (wherein the arrayed molecules are ligands, agonists or antagonists of said receptors).

One useful feature of biochips is the manner in which the arrayed biomolecules are attached to the surface of the biochip. Conventionally such procedures involve multiple reaction steps, often requiring chemical modification of the solid support itself. Even in embodiments comprising absorption matrices, such as hydrogels, present on a solid support, chemical modification of the gel polymer is necessary to provide a chemical functionality capable forming a covalent bond with the biomolecule. The efficiency of the attachment chemistry and strength

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of the chemical bonds formed are critical to the fabrication and ultimate performance of the microarray.

Polymeric hydrogels and gel pads are used as binding layers to adhere to surfaces biological molecules including, but not limited to, proteins, peptides, oligonucleotides, polynucleotides, and larger nucleic acid fragments. The oligonucleotide probes may be bound to the surface of a continuous layer of the hydrogel, or to an array of gel pads. The gel pads comprising biochips for use with the apparatus of the present invention are conveniently produced as thin sheets or slabs, typically by depositing a solution of acrylamide monomer, a crosslinker such methylene bisacrylamide, and a catalyst such as N, N, N', N' - tetramethylethylendiamine (TEMED) and an initiator such as ammonium persulfate for chemical polymerization, or 2,2-dimethoxy-2-phenyl-acetophone (DMPAP) for photopolymerization, in between two glass surfaces (e.g., glass plates or microscope slides) using a spacer to obtain the desired thickness of the polymeric gel. Generally, the acrylamide monomer and crosslinker are prepared in one solution of about 4-5% acrylamide (having an acrylamide/bisacrylamide ratio of 19/1) in water/glycerol, with a nominal amount of initiator added. The solution is polymerized and crosslinked either by ultraviolet (UV) radiation (e.g., 254 nm for at least about 15 minutes, or other appropriate UV conditions, collectively termed "photopolymerization"), or by thermal initiation at elevated temperature (e.g., typically at about 40° C). Following polymerization and crosslinking, the top glass slide is removed from the surface to uncover the gel. The pore size (and hence the "sieving properties") of the gel is controlled by changing the amount of crosslinker and the percent solids in the monomer solution. The pore size also can be controlled by changing the polymerization temperature.

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In the fabrication of polyacrylamide embodiments of the polymeric hydrogel arrays of the invention (i.e., patterned gels) used as binding layers for biological molecules, the acrylamide solution typically is imaged through a mask during the UV polymerization/crosslinking step. The top glass slide is removed after polymerization, and the unpolymerized monomer is washed away (developed) with water, leaving a fine feature pattern of polyacrylamide hydrogel, which is used to produce the crosslinked polyacrylamide hydrogel pads. Further, in an application of lithographic techniques known in the semiconductor industry, light can be applied to discrete locations on the surface of a polyacrylamide hydrogel to activate these specified regions for the attachment of an oligonucleotide, an antibody, an antigen, a hormone, hormone receptor, a ligand or a polysaccharide on the surface (e.g., a polyacrylamide hydrogel surface) of a solid support (*see, for example,* International Application, Publication No. WO 91/07087, incorporated by reference).

For hydrogel-based arrays using polyacrylamide, biomolecules (such as oligonucleotides) are covalently attached by forming an amide, ester or disulfide bond between the biomolecule and a derivatized polymer comprising the cognate chemical group. Covalent attachment of the biomolecule to the polymer is usually performed after polymerization and chemical cross-linking of the polymer is completed

Alternatively, oligonucleotides bearing 5'-terminal acrylamide modifications can be used that efficiently copolymerize with acrylamide monomers to form DNA-containing polyacrylamide copolymers (Rehman *et al.*, 1999, *Nucleic Acids Research* 27: 649-655). Using this approach, stable probe-containing layers can be fabricated on supports (e.g., microtiter plates and silanized glass) having exposed acrylic groups. This approach has made available the commercially marketed "Acrydite™" capture probes (available from Mosaic Technologies,

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Boston, MA). The Acrydite moiety is a phosporamidite that contains an ethylene group capable of free-radical copolymerization with acrylamide, and which can be used in standard DNA synthesizers to introduce copolymerizable groups at the 5' terminus of any oligonucleotide probe.

With reference to the illustration provided in Figure 1, the invention provides a hybridization chamber **10** comprising a biochip, which comprises a substrate **11** having a first surface **12** and a second surface **13** opposite thereto, and a flexible layer **16** affixed to the first substrate surface **12** by an adhesive layer **15**. On the first surface **12** is an area **14** bounded by adhesive layer **15** an completely covered by flexible layer **16**. Flexible layer **16**, adhesive layer **15**, and first substrate surface **12** further define a volume **25**. The ratio of volume **25** to area **14** is preferably from about 0.025 $\mu L/mm^2$ to about 0.25 $\mu L/mm^2$, more preferably from about 0.1 $\mu L/mm^2$ to about 0.25 $\mu L/mm^2$, and most preferably from about 0.1 $\mu L/mm^2$ to about 0.2 $\mu L/mm^2$.

As shown in Figure 3, between flexible layer **16** and first substrate surface **12** in area **14** is positioned a multiplicity of biomolecules. In a preferred embodiment, the multiplicity of biomolecules comprises an array **17** of biomolecules, which is preferably affixed to first substrate surface **12**. Array **17** preferably further comprises gel pads **22**. In an alternate preferred embodiment, array **17** is deposited on a continuous layer of polyacrylamide. Figure 2 provides an exploded cross-sectional view of a portion of array **17** illustrating the gel pads **22**. Each gel structure **22** is preferably cylindrical, most preferably having about a 113 micron diameter and about a 25 micron thickness. The distance between each site within each array **17** is most preferably about 300 microns.

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A layer of a water-soluble compound **28** having a melting point of about 30 to about 60°C, more preferably of about 35 to about 50°C, and most preferably of about 35 to about 45°C is deposited in volume **25** bounded by first substrate surface **12**, flexible layer **16**, and adhesive layer **15**. Preferably, the water-soluble compound is biocompatible, does not stick to flexible layer **16**, and serves to prevent mechanical damage to gel pads **22**. In a preferred embodiment, the compound is polyethylene glycol, most preferably polyethylene glycol 600. The compound **28** is deposited so that the entire volume **25**, with the exception of that portion of volume **25** occupied by array **17**, comprises compound **28**.

Array **17** can be positioned on surface **12** by providing markings, most preferably holes or pits in surface **12**, that act as fiducials or reference points on surface **12** for accurate placement of array **17**. The presence of said fiducials is particularly advantageous in embodiments comprising a multiplicity of arrays **17** in one or a multiplicity of areas **14** on surface **12**, wherein accurate placement of said arrays is required for proper spacing and orientation of the arrays on the hybridization chamber.

In preferred embodiments, a first and second port **19** and **20** extend through flexible layer **16**, as shown in Figure 7. The first port **19** serves as an input port and is positioned in flexible layer **16** so that the first opening **29** is provided within the area **14** bounded by adhesive layer **15** on first surface **12**. Second port **20** serves as an outlet port and is positioned in flexible layer **16** so that the first opening **31** opens within area **14** bounded by the adhesive layer **15** on the first surface **12**. The openings of ports **19** and **20** may be covered with a removable and replaceable cover **21**. In preferred embodiments, replaceable cover **21** is a stopper, a gasket, or tape, most preferably a foil tape.

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In these embodiments, one or more first notches **70** are cut into the first adhesive layer **15** such that the first notches **70** are in direct communication with the area **14** on first substrate surface **12** bounded by the first adhesive layer **15**. Second notches **72** are cut into the flexible layer **16** in positions corresponding to the size and position of first notches **70** in adhesive layer **15**, thus forming one or more ports. In a particularly preferred embodiment, a ring of adhesive **74** is deposited around the perimeter of each second notch **72**, such that the inner perimeter of adhesive ring **74** is coextensive with the outer perimeter of second notch **72**. Preferably, first and second notches **70** and **72** are circular in shape, and have a diameter that is equal to the inner diameter of adhesive ring **74**. Preferably the inner diameter and outer diameter of adhesive ring **74** are selected to form a tight seal with the tip end of a pipette. In an alternate preferred embodiment, a second layer of adhesive **76** is deposited on the portions of flexible layer **16** not covering the area **14** on first substrate surface **12** and not defining first and second ports **19** and **20**. In this embodiment, the apparatus further comprises a label layer **57** that is die cut in the same manner as the first adhesive layer **15** to form windows **58** that correspond in location to areas **14** on first substrate surface **12**, and which is applied to second adhesive layer **76**. In this embodiment, one or more third notches **78** are cut into second adhesive layer **76**, such that third notches **78** correspond in shape, size, and position to first and second notches **70** and **72**. Fourth notches **80**, having a shape and position corresponding to first, second and third notches **70, 72** and **78**, are cut into label layer **57**. The diameter of fourth notches **80** is preferably greater than the diameter of first, second and third notches **70, 72** and **78**, such that after the apparatus is assembled a portion of second adhesive layer **76** is exposed by fourth notch **80**. Preferably the exposed portion of second adhesive layer **76** corresponds to the shape and size of a pipette tip.

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In alternative embodiments of the apparatus, first and second ports **19** and **20** extend through substrate **11**, rather than through flexible layer **16**. Illustrative embodiments are described in co-owned and co-pending U.S. Application No. 09/464,490, incorporated by reference herein.

In preferred embodiments of the apparatus, area **14** on first substrate surface **12** is square or rectangular with two rounded edges at diagonally opposite corners of are **14** and two 90 degree angles at the remaining two diagonally opposite corners of area **14**. Preferably, when first and second ports **19** and **20** extend through flexible layer **16**, first notches **70** in first adhesive layer **15** are cut at the sharp edges of area **14**, as shown in Figure 7. These embodiments are particularly preferred as they comprise geometries that eliminate corners and therefore are useful in the prevention of bubble formation in area **14**.

Substrate **11** is fabricated from any solid supporting substance, including but not limited to plastics, metals, ceramics, and glasses. Most preferably, substrate **11** is made from silicon or glass (for accuracy and stiffness), molded plastics (which reduce cost of manufacture and thermal inertia), or ceramics (for the incorporation of microfluidic elements including integrated heating elements). Most preferably, the substrate is glass.

Adhesive layer **15** is prepared using an adhesive suitable for forming a water-tight bond between substrate **11** and flexible layer **16**, including, but not limited to, high temperature acrylics, rubber-based adhesives, and silicone-based adhesives. The shape of adhesive layer **15** is configured to contain array **17**. Adhesive layer **15** can be deposited on first substrate surface **12** in a pattern to produce area **14** in any desired shape, and is most preferably deposited to define an ellipsoid area **14**. Adhesive layer **15** can be deposited using inkjet printing or offset printing methods, or by die cutting the desired shapes from a sheet of adhesive material. In addition, a

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substantial portion of first surface **12** can be covered with adhesive and portions of the substrate that are not desired to retain adhesive properties can be hardened preferentially, for example, by ultraviolet curing. In these embodiments, portions retaining adhesive properties can be defined using a mask and thereby retain adhesive properties necessary to affix flexible layer **16** to surface **12**. In embodiments using the die cut adhesive material, the adhesive material is preferably a doublesided adhesive tape, and more preferably a double sided adhesive tape having no carrier. Adhesive layer **15** is most preferably set down in a layer between 1 and 100 μm thick, more preferably between 25 and 50 μm thick, and most preferably about 50 μm thick.

Flexible layer **16** is made of any flexible solid substance, including but not limited to plastics, including polypropylene, polyethylene, and polyvinylidene chloride (sold commercially as Saran® wrap) plastics, rubbers, including silicone rubbers, high temperature polyesters, and porous Teflon®. Flexible layer **16** is preferably biocompatible and preferably has low permeability in order to prevent evaporation of water from the volume contained between the flexible layer and the substrate. Flexible layer **16** also preferably is optically clear and should be able to withstand temperatures of between 50 and 95°C for a period of between 8 and 12 hours without shrinkage. In a preferred embodiment, the flexible layer is a gas permeable membrane. Flexible layer **16** preferably covers an area of from about 5 mm^2 to about 1400 mm^2, more preferably from about 5 mm^2 to about 600 mm^2, and most preferably from about 100 mm^2 to about 600 mm^2.

In preferred embodiments, as shown in Figure 5, the invention further comprises a label layer **57** that is die cut in the same manner as the adhesive to form windows **58** that correspond in location to areas **14** on first substrate surface **12**. The label layer is preferably a thick film having

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a layer of adhesive, and most preferably is an Avery laser label. The label layer is applied to the outer surface of the flexible layer, preferably by vacuum lamination.

Array **17** contained in area **14** on first substrate surface **12** is covered with a water-soluble compound **28**, which protects and seals the biochip prior to use and prevents degradation or other damage to the array. Any water-soluble compound **28** having a melting point of about 30°C to about 60°C, more preferably of about 35°C to about 50°C, and most preferably of about 35°C to about 45°C is advantageously used in filling volume **25** between array **17** and flexible layer **16**. Preferably, the compound is polyethylene glycol, most preferably polyethylene glycol 600. It is a particularly preferred feature of hybridization chamber **10** of the invention that water-soluble compound **28** fills the entirety of the volume **25** and more preferably also fills at least a portion of input port **19**. This prevents formation of air bubbles in volume **25** because compound **28** is first melted, then carefully mixed with hybridization fluid **26** within volume **25** using a roller **40** without producing air bubbles in hybridization fluid **26**. The lack of air bubbles in volume **25** minimizes artifactual signals detected by a scanner **36** or a light pipe **37**.

Ports and holes can be produced in substrate **11** by diamond drilling in glass embodiments of substrate **11** or by stamping or molding in plastic embodiments thereof. This facilitates standardization of the hybridization chamber dimensions, for example, by producing substrates where the first and second ports **19** and **20** are produced in a single operation. Both the substrate **11** and the removable cover **21** can be set down as strips or large sheets, and can be rolled to avoid trapping air bubbles. Flexible layer **16** can be applied by vacuum lamination to avoid trapping air, or can be deposited by spinning or flowing liquid plastic over substrate **11** after treatment with adhesive **15** and water-soluble compound **28**, followed by curing the flexible

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layer in place. Individual hybridization chambers **10** can be produced in stacks using, for example, a diamond saw as shown in Figure 6.

Figure 6 illustrates a preferred arrangement for manufacturing hybridization chamber **10**, wherein alternating layers of flexible layer **16**, adhesive layer **15**, uncut substrate **11**, and removable cover **21** are laid down, and hybridization chambers are produced by cutting the stacked layers, for example, with a diamond saw or any appropriate manufacturing tool. The sealed volumes **25** protect the arrays **17** from debris produced during the cutting process.

Alternative embodiments of the hybridization chamber **10** of the invention encompass a multiplicity of arrays **17** confined in a multiplicity of areas **14** underneath flexible layer **16**, each area comprising an array **17** and being supplied with first port **19** and, optionally, second port **20**. In these embodiments, adhesive layer **15** is deposited on first substrate surface **12** in a pattern that defines each of areas **14**, and flexible layer **16** is applied to adhesive layer **15** to encompass areas **14** on said surface.

In certain embodiments of the invention, hybridization chamber **10** is produced containing array **17** or a multiplicity of arrays **17** as disclosed herein, wherein the chamber is provided ready-to-use by the addition of hybridization fluid **26** comprising one or a multiplicity of target molecules. In alternative embodiments, hybridization chamber **10** is provided without array **17**, and allows for insertion thereof by a user. In these embodiments, at least one edge of flexible layer **16** is not adhered to first substrate surface **12**.

In the use of the hybridization chamber **10** of the invention, an amount of a hybridization fluid **26**, most preferably comprising a biological sample containing a target nucleic acid, is added to the hybridization chamber through first port **19**. Before application of the hybridization fluid **26** to the chamber, volume **25** is most preferably heated to a temperature greater than or

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equal to the melting temperature of water-soluble compound **28**. When melted, hybridization fluid **26** can be added to the chamber and mixed with the water-soluble compound, as shown in Figure 1B. Preferably, water-soluble compound **28** does not affect hybridization in the chamber. More preferably, the amount of compound **28** is chosen such that hybridization efficiency is improved when compound **28** is mixed with sample fluid **26**.

In embodiments of the chamber comprising first port **19** but not second port **20**, the hybridization fluid is preferably introduced into the chamber after compound **28** is melted, and then the fluid is cycled into and out of the chamber using, most preferably, a pipette, until fluid **26** and compound **28** are fully mixed, and the hybridization fluid evenly distributed over the surface of array **17**, or mixed into gel pads **22** comprising certain embodiments of said arrays. Alternatively, hybridization fluid **26** is evenly distributed over the surface of array **17**, or mixed into gel pads **22** by physically manipulating flexible layer **16**, as more fully described below. In these embodiments, hybridization fluid **26** is removed after hybridization is completed, as shown in Figure 9, and array **17** is washed by the cycling a sufficient volume of a wash solution **27** into and out of the chamber, most preferably using a pipette.

In embodiments of the chamber comprising both first port **19** and second port **20**, the hybridization fluid is preferably introduced into the chamber after compound **28** is melted, and then the fluid is cycled into and out of the chamber using, most preferably, at least one pipette, until fluid **26** and compound **28** are fully mixed, and the hybridization fluid evenly distributed over the surface of array **17**, or mixed into gel pads **22** comprising certain embodiments of said biochips. Hybridization is then performed by incubating the chamber for a time and at a temperature sufficient for hybridization to be accomplished. Hybridization fluid **26** is removed after hybridization has been completed using outlet port **20**, and the biochip washed by the

addition and cycling of a sufficient volume of a wash solution **27** into and out of the chamber, most preferably using a pipette. In these embodiments, the wash solution can also be continuously provided by addition through the input port and removal through the output port. In certain embodiments, the biochip containing the hybridized array is removed from the chamber for development or further manipulations as required. In preferred embodiments, the biochip containing the hybridized array is analyzed *in situ* as described below.

Figure 1B illustrates an advantageous embodiment of hybridization chamber **10** of the invention, further comprising a heating element **33**. Most advantageously, heating element **33** has a heating surface **34** adapted to the shape of hybridization chamber **10** to substantially cover the area **14** under flexible layer **16**. Heating element **33** is any suitable heating means, including but not limited to resistance heaters, thermoelectric heaters, or microwave absorbing heaters.

The hybridization chamber **10** of the invention also advantageously comprises a thermocouple **35** or other temperature-sensing or measuring element to measure the temperature of hybridization fluid **26** or chamber **10**. These temperature-sensing elements advantageously are coupled with heating element **33** to control hybridization fluid **26** and wash solution **27** temperature, and can be used to calibrate other elements, such as scanning devices **36** as described below that may be sensitive to temperature.

In certain embodiments of the invention, positive hybridization is detected visually, *i.e.*, by the production of a dye or other material that reflects visible light at sites on biochip **18** where hybridization has occurred. In these embodiments, the dye or other material is most preferably produced enzymatically, for example, using a hybridization-specific immunological reagent such as an antibody linked to an enzyme that catalyzes the production of the dye. Visual inspection

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can be used to detect sites of positive hybridization. More preferably, the biochip containing the hybridized array is scanned using scanner **36** as disclosed more fully below.

Positive hybridization on biochip **18** most preferably is detected by fluorescence using labeled target molecules in a biological sample, or by including intercalating dyes in the hybridization fluid **26** that fluoresce when bound by a hybridized DNA duplex and illuminated by light at a particular wavelength. Suitable intercalating dyes include, but are not limited to, ethidium bromide, Hoechst DAPI, and Alexa Fluor dyes. Suitable fluorescence labels include, but are not limited to, fluorescein, rhodamine, propidium iodide, and Cy3 and Cy5 (Amersham), that can be incorporated into target molecules, for example, *in vitro* amplified fragments using labeled oligonucleotide primers.

Figures 10A-10C illustrate an embodiment of the invention comprising a scanner **36**, which is advantageously positioned over (or beneath) flexible layer **16** and moves from one end of area **14** to the opposite end, sequentially illuminating area **14** and array **17** positioned thereupon. Prior to analysis of the hybridized array, all fluid is removed from volume **25** such that flexible layer **16** is in contact with array **17**. Scanner **36** then excites the fluorescent dye, preferably with short wavelength light, most preferably light with a wavelength between 250 nm and 600 nm. Scanner **36** then collects the emitted light from a specific area. The amount of light emitted is then used to determine the amount of fluorescent dye present in the area, and hence the amount of labeled target.

Particular embodiments of scanners and scanning devices **36** are shown in Figures 11A through 11E. It is a particularly advantageous feature of hybridization chamber **10** that flexible layer **16** is translucent to suitable wavelengths of light, including light in the ultraviolet and visible portion of the spectrum. An additional advantageous feature of hybridization chamber **10**

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is that flexible layer **16**, which is very thin, is immediately adjacent to and in contact with biochip **18** after hybridization fluid **26** or wash fluid **27** is removed from the chamber. This combination of features reduces or eliminates free surface reflections, internal reflection of illumination from the scanner, and dispersion or scattering of illuminating light, thereby optimizing the amount of incident light that illuminates array **17**. This arrangement is also more economical than in existing apparatus as it minimizes the need for highly polished, low scattering surfaces or complex or expensive lenses, and eliminates problems associated with focus and depth-of-field in more complex optical detectors.

In other embodiments, a light pipe **37** contacts the surface of flexible layer **16** that is immediately adjacent to and in contact with the surface of array **17**, as shown in Figure 11B. In these embodiments, both illuminating and emitted light are conveyed and collected by light pipe **37**. The pipe is designed to be slightly flexible so as to adapt to the contoured surface of flexible layer **16**. Light pipe **37** contacts flexible layer **16** that contacts array **17**, thereby permitting contacts free of surface reflections even under circumstances where array **17** or light pipe **37** has localized variations in height. Advantageously, light pipe **37** has a larger surface area than array **17**, so that the maximum amount of illuminating light is delivered to array **17**, and the maximum amount of emitted light from array **17** is collected by light pipe **37**. A further advantage of light pipe **37** is that it enables detection of bubbles formed in hybridization fluid **26** or wash buffer **27**, which detection can be used as a signal for roller **40** to address flexible layer **16** to remove such bubbles. Removing bubbles in hybridization fluid **26** or wash buffer **27** reduces the frequency of non-specific binding and artifactual signals detected by scanner **36**.

In additional embodiments of the invention, the area **14** defined by adhesive layer **15** further comprises a reflective layer **38** that substantially covers the entirety of the area **14** and is

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positioned between array **17** and the first substrate surface **12**. In preferred embodiments, reflective layer **38** comprises aluminum, gold, silver, or platinum. In these embodiments, the amount of the light signal reflected or transmitted to the light-detecting portion of scanner **36** is increased up to four-fold. In further advantageous embodiments of the invention, reflective layer **38** is a metal film resistor or an RF induction heater. In these embodiments, reflective **38** layer can heat the slide without requiring additional heating elements **33**. This is a particularly desirable feature in hand-held embodiments of the hybridization chamber **10** of the invention.

If required, a passivation later **39** can be applied on top of reflective layer **38**. Preferably, passivation layer **39** is a layer of parylene a few microns thick that is applied by evaporation. The amount of illumination required, and hence the amount of power needed to operate scanner **36** is reduced in these embodiments, which are particularly suited to battery-operated embodiments such as hand-held devices to improve useful battery life. Furthermore, passivation layer **39** reduces artifactual signals in the light emission data by obscuring objects that it covers.

Hybridization chamber **10** is preferably supplied with a roller **40** in removable contact with flexible layer **16** and that can be moved longitudinally across areas **14** on first substrate surface **12**. In preferred embodiments, the surface of roller **40** comprises a textured pattern **41**, most preferably a spiral pattern, that permits the roller to efficiently mix hybridization fluid and wash solution across area **14** and array **17**. One advantageous arrangement of roller **40** and hybridization chamber **10** is shown in Figure 11E. As shown in the Figure, roller **40** can be advantageously connected to a movable arm **42** that can be positioned to place roller **40** in contact with flexible layer **16** when in a first position, and can be moved to a second position in which roller **40** is not in contact with flexible layer **16**. Most preferably, movable arm **42** has a

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pivot point **44** and movement about said pivot point is preferably controlled by a solenoid. In addition to movement of roller **40** in contact with and away from hybridization chamber **10**, either roller **40** or hybridization chamber **10**, or both, are movable in a longitudinal direction to enable roller **40** to mix hybridization fluid **26** or wash solution **27** inside volume **25** bounded by flexible layer **16**, adhesive layer **15**, and first substrate surface **12** in area **14** containing array **17**. In embodiments comprising a multiplicity of areas **14** containing a multiplicity of arrays **17**, roller **40** is positioned to move longitudinally across each of the multiplicity of areas **14** to mix hybridization fluid **26** or wash solution **27** in each of the volumes **25** containing arrays **17**.

In additional embodiments, a sample preparation chip **45**, comprising a port **46**, as shown in Figures 12A through 12C, can be attached to hybridization chamber **10**. Most preferably, port **46** in sample preparation chip **45** is aligned with first port **19** in hybridization chamber **10** to permit efficient transfer of sample to volume **25**. Additional fiducial references can be used to accurately align hybridization chamber **10** and sample preparation chip **45**. Since access to first port **19** is through second substrate surface **13**, the array can be scanned without interference from the attached sample preparation chip. In alternative embodiments of the invention, sample preparation chip **45** may be bound to second substrate surface **13** (Figure 12B) or formed as an integral part of substrate **11** (Figure 12C).

A preferred embodiment of hybridization chamber **10** of the invention is a hand-held embodiment as shown in Figures 10A-10C, further comprising a scanner **36**. In these embodiments, hand-held device **47** comprises a base **48**, a lid **49** and a carriage **50** embodying roller **40**, scanner **36**, heating element **33** and thermocouple **35**. Carriage **50** is illustrated in Figure 11A. Device **47** comprises a compartment **51** for positioning hybridization chamber **10** in proximity to carriage **50**. Carriage **50** is provided with moving means for moving roller **40**,

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scanner **36** and heating element **33** relative to hybridization chamber **10** as required for operation as described above. Carriage **50** and lid **49** are arranged to permit a user to introduce and remove hybridization fluid **26** and wash solution **27** into the chamber through first port **19** and second port **20** as required. Alternatively, device **47** further comprises fluidic connections **52** to each of the first and second ports to provide for sample introduction and array washing after hybridization of the sample thereto. Device **47** is most preferably operated by battery, although AC adapters are also advantageously encompassed by the description of the device herein. In further preferred embodiments, lid **49** further comprises a display **56** for displaying the results of the analysis.

The Examples that follow are illustrative of specific embodiments of the invention and various uses thereof. They are set forth for explanatory purposes only, and are not to be taken as limiting the invention.

EXAMPLE 1

ASSEMBLY OF A HYBRIDIZATION CHAMBER

The process of assembling a chamber according to the present invention is illustrated in Figure 13.

A die cutter was used to cut four ellipsoidal holes in a layer of 502FL ultra-clean laminating adhesive film (3M). A similar pattern was punched into an Avery laser label 5663 for use as a frame and label layer. Meanwhile, a sheet of polyvinylidene chloride film was stretched over a stainless steel frame and annealed for 30 minutes at 100°C. The Avery label was applied to one side of the polyvinylidine chloride film by vacuum laminating the label in a vacuum lamination press. A vacuum of 15 psi was applied for 30 seconds, and mechanical pressure of 15

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psi was maintained for 1 minute after release of the vacuum. The adhesive was then applied to the opposite side of the polyvinylidene chloride film using the same process as for the label.

The adhesive coated film was then applied to a glass slide that had previously been prepared. The arrays of oligonucleotide probes and gel pads were positioned on the glass slide using standard methods. Ports were drilled into the slide using a diamond drill. A vacuum lamination press was used to affix the polyvinylidene chloride film to the slide. A vacuum of 15 psi was maintained for 1 minute, and then mechanical pressure of 15 psi was maintained for an additional minute.

The individual chambers were then filled with polyethylene glycol 600 using a 10 μL pipette tip. A layer of 3M 7350 polyester tape was then applied to the slide to seal off the ports.

EXAMPLE 2

ASSEMBLY OF A TOP-LOADING HYBRIDIZATION CHAMBER

A die cutter was used to cut four ellipsoidal holes in a layer of 502FL ultra-clean laminating adhesive film (3M). A similar pattern was punched into an Avery laser label 5663 for use as a frame and label layer. Meanwhile, a sheet of polyvinylidene chloride film was stretched over a stainless steel frame and annealed for 30 minutes at 100°C. The Avery label was applied to one side of the polyvinylidine chloride film by vacuum laminating the label in a vacuum lamination press. A vacuum of 15 psi was applied for 30 seconds, and mechanical pressure of 15 psi was maintained for 1 minute after release of the vacuum. The adhesive was then applied to the opposite side of the polyvinylidene chloride film using the same process as for the label.

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The adhesive coated film was then applied to a glass slide that had previously been prepared. The arrays of oligonucleotide probes and gel pads were positioned on the glass slide using standard methods. A vacuum lamination press was used to affix the polyvinylidene chloride film to the slide. A vacuum of 15 psi was maintained for 1 minute, and then mechanical pressure of 15 psi was maintained for an additional minute.

The individual chambers were then filled with polyethylene glycol 600 using a 10 μL pipette tip. A layer of 3M 7350 polyester tape was then applied to the slide to seal off the ports.

It should be understood that the foregoing disclosure emphasizes certain specific embodiments of the invention and that all modifications or alternatives equivalent thereto are within the spirit and scope of the invention as set forth in the appended claims.

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